

WO 99/50410

PCT/AU99/00220

- 99 -

CLAIMS:

1. An isolated nucleic acid molecule comprising a sequence of nucleotides encoding, or a complementary to a nucleotide sequence encoding, a protein or derivative or homolog thereof wherein said sequence is expressed in heart muscles or a derivative, homolog, analog, chemical equivalent or mimetic of said nucleic acid molecule.
2. An isolated nucleic acid molecule according to claim 1 wherein said protein comprises the amino acid sequence substantially as set forth in SEQ ID NO:2 or a derivative, homolog or mimetic thereof or having at least about 45% or greater similarity to SEQ ID NO:2 or a derivative, homolog, analog, chemical equivalent or mimetic of said nucleic acid molecule.
3. An isolated nucleic acid molecule according to claim 1 comprising a nucleotide sequence substantially as set forth in SEQ ID NO:1 or a derivative, homolog or mimetic thereof or capable of hybridizing to SEQ ID NO:1 under low stringency conditions or a derivative, homolog, analog, chemical equivalent or mimetic of said nucleic acid molecule.
4. An isolated nucleic acid molecule according to claim 3 which further encodes an amino acid sequence corresponding to an amino acid sequence substantially as set forth in SEQ ID NO:2 or a sequence having at least about 45% similarity to SEQ ID NO:2.
5. An isolated nucleic acid molecule according to claim 2 or 3 substantially as set forth in SEQ ID NO:1.
6. An isolated nucleic acid molecule according to claim 1 wherein said protein has the characteristics of Csl or a functional equivalent thereof.

WO 99/50410

PCT/AU99/00220

- 100 -

7. An isolated nucleic acid molecule according to claim 1 wherein said protein comprises the amino acid sequence substantially as set forth in SEQ ID NO:4 or a derivative, homolog or mimetic thereof having at least about 45% or greater similarity to SEQ ID NO:4 or a derivative, homolog, analog, chemical equivalent or mimetic of said nucleic acid molecule.
8. An isolated nucleic acid molecule according to claim 1 comprising a nucleotide sequence substantially as set forth in SEQ ID NO:3 or a derivative, homolog or mimetic thereof or capable of hybridising to SEQ ID NO:3 under low stringency conditions or a derivative, homolog, analog, chemical equivalent or mimetic of said nucleic acid molecule.
9. An isolated nucleic acid molecule according to claim 8 which further encodes an amino acid sequence corresponding to an amino acid sequence substantially as set forth in SEQ ID NO:4 or a sequence having at least about 45% similarity to SEQ ID NO:4.
10. An isolated nucleic acid molecule according to claim 7 or 8 substantially as set forth in SEQ ID NO:3.
11. An isolated nucleic acid molecule according to claim 1 wherein said protein comprises the amino acid sequence substantially as set forth in SEQ ID NO:5 or a derivative, homolog or mimetic thereof or having at least about 45% or greater similarity to SEQ ID NO:5 or a derivative, homolog, analog, chemical equivalent or mimetic of said nucleic acid molecule.
12. An isolated nucleic acid molecule according to claim 1 comprising a nucleotide sequence comprising exon regions of which five comprise

Exon 1 comprising a nucleotide sequence corresponding to the nucleotide sequence substantially as set forth in SEQ ID NO:6;

05647049-043601

RB3

WO 99/50410

PCT/AU99/00220

- 101 -

Exon 2 comprising a nucleotide sequence corresponding to the nucleotide sequence substantially as set forth in SEQ ID NO:7;

Exon 3 comprising a nucleotide sequence corresponding to the nucleotide sequence substantially as set forth in SEQ ID NO:8;

Exon 4 comprising the nucleotide sequence corresponding to the nucleotide sequence substantially as set forth in SEQ ID NO:9; and

Exon 5 comprising a nucleotide sequence corresponding to the nucleotide sequence substantially as set forth in SEQ ID NO:10,

or capable of hybridizing to a genomic sequence comprising said exon regions under low stringency conditions or a derivative, homolog, analog, chemical equivalent or mimetic of said nucleic acid molecule.


13. An isolated nucleic acid molecule according to claim 12 wherein said nucleotide sequence corresponds to the gene maps set forth in Figure 2.

14. An isolated nucleic acid molecule according to claim 12 ~~or 13~~ which further encodes an amino acid sequence corresponding to an ~~amino~~ acid sequence set forth in SEQ ID NO:4 or a derivative, homolog or mimetic thereof or a sequence having at least about 45% similarity to SEQ ID NO:4 or a derivative, homolog, analog, chemical equivalent or mimetic of said nucleic acid molecule.

15. An isolated protein or a derivative, homolog, analog, chemical equivalent or mimetic thereof wherein said protein is expressed in muscle cells.

16. An isolated protein according to claim 15 comprising an amino acid sequence substantially as set forth in SEQ ID NO:2 or a derivative, homolog or mimetic thereof or having at least about 45% or greater similarity to SEQ ID NO:2 or a derivative, homolog, analog, chemical equivalent or mimetic of said protein.

09647049.02601



WO 99/50410

PCT/AU99/00220

- 102 -

a

17. An isolated protein according to claim 15 ~~or 16~~ encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:1 or a derivative, homolog or mimetic thereof or capable of hybridizing to SEQ ID NO:1 under low stringency conditions or a derivative, homolog, analog, chemical equivalent or mimetic of said protein.

a

18. An isolated protein according to claim 16 ~~or 17~~ substantially as set forth in SEQ ID NO:2.

19. An isolated protein according to claim 15 wherein said protein has the characteristics of Csl or a functional equivalent thereof.

20. An isolated protein according to claim 15 comprising an amino acid sequence substantially as set forth in SEQ ID NO:4 or a derivative, homolog or mimetic thereof or having at least about 45% or greater similarity to SEQ ID NO:4 or a derivative, homolog, analog, chemical equivalent or mimetic of said protein.

a

21. An isolated protein according to claim 15 ~~or 20~~ encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:3 or a derivative, homolog or mimetic thereof or capable of hybridizing to SEQ ID NO:3 under low stringency conditions or a derivative, homolog, analog, chemical equivalent or mimetic of said protein.

a

22. An isolated protein according to claim 15 ~~or 20~~ encoded by a nucleotide sequence comprising exon regions of which five comprise

Exon 1 comprising a nucleotide sequence corresponding to the nucleotide sequence substantially as set forth in SEQ ID NO:6;

Exon 2 comprising a nucleotide sequence corresponding to the nucleotide sequence substantially as set forth in SEQ ID NO:7;

Exon 3 comprising a nucleotide sequence corresponding to the nucleotide sequence substantially as set forth in SEQ ID NO:8;

WO 99/50410

PCT/AU99/00220

- 103 -

Exon 4 comprising the nucleotide sequence corresponding to the nucleotide sequence substantially as set forth in SEQ ID NO:9; and
Exon 5 comprising a nucleotide sequence corresponding to the nucleotide sequence substantially as set forth in SEQ ID NO:10,

or capable of hybridizing to a genomic sequence comprising said exon regions under low stringency conditions or a derivative, homolog, analog, chemical equivalent or mimetic of said protein.

23. An isolated protein according to claim 22 wherein said nucleotide sequence corresponds to the gene map as set forth in Figure 2.

24. An isolated protein according to claim 20, ~~21, 22 or 23~~ substantially as set forth in SEQ ID NO:4.

25. An isolated protein according to claim 15 comprising an amino acid sequence substantially as set forth in SEQ ID NO:5 or a derivative, homolog or mimetic thereof or having at least about 45% or greater similarity to SEQ ID NO:5 or a derivative, homolog, analog, chemical equivalent or mimetic of said protein.

26. An isolated protein according to ~~any one of claims 15-25~~ claim 15 which protein is a homodimer.

27. An isolated protein according to ~~any one of claims 15-25~~ claim 15 which protein is a heterodimer.

28. A method for modulating expression of *Csl* in a mammal, said method comprising contacting the *Csl* gene with an effective amount of an agent for a time and under conditions sufficient to modulate expression of *Csl*.

WO 99/50410

PCT/AU99/00220

- 104 -

29. A method for modulating the functional activity of Csl in a mammal, said method comprising administering to said mammal a modulating effective amount of an agent for a time and under conditions sufficient to increase or decrease Csl activity.

30. A method for modulating muscle cell functional activity in a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the expression of a nucleotide sequence encoding Csl or sufficient to modulate the activity of Csl.

31. A method of modulating muscle cell functional activity in a mammal said method comprising administering to said mammal an effective amount of a protein according to ~~any one of claims 15-25~~ ^{claim 15} or a derivative, homolog, analog, chemical equivalent or mimetic thereof for a time and under conditions sufficient to modulate the functional activity of said muscle cell.

32. A method according to claim 31 wherein said protein interacts, binds or otherwise associates with an EF-Hand target.

33. A method according to claim 32 wherein said EF-Hand target is the IQ site on the myosin heavy chain or a calcineurin-A catalytic subunit.

34. A method of modulating muscle cell functional activity in a mammal said method comprising administering to said mammal an effective amount of a nucleic acid molecule according to ~~any one of claims 1-14~~ ^{claim 1} or a derivative, homolog, analog, chemical equivalent or mimetic thereof for a time and under conditions sufficient to modulate the functional activity of said muscle cell.

35. A method according to claim 34 wherein the expression product of said nucleic acid molecule interacts, binds or otherwise associates with an EF-Hand target.

a 09647039-032604
a/b7


a 4888

WO 99/50410

PCT/AU99/00220


- 105 -

36. A method according to claim 35 wherein said EF-Hand target is the IQ site on a myosin heavy chain or a calcineurin-A catalytic subunit.

a  37. A method of modulating cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of a protein according to ^{claim 15} ~~any one of claims 15-25~~ or a derivative, homolog, analog, chemical equivalent or mimetic thereof for a time and under conditions sufficient to modulate the activity of one or more components of a calcineurin-dependent signalling pathway.

38. A method according to claim 37 wherein said cellular functional activity is muscle cell functional activity.

39. A method according to claim 37 ~~or 38~~ wherein said component is the calcineurin-A catalytic subunit.

a  40. A method of modulating cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of a nucleic acid molecule according to ^{claim 1} ~~any one of claims 1-14~~ or a derivative, homolog, analog, chemical equivalent or mimetic thereof for a time and under conditions sufficient to modulate the activity of one or more components of a calcineurin-dependent signalling pathway.

41. A method according to claim 40 wherein said cellular functional activity is muscle cell functional activity.

a 42. A method according to claim 40 ~~or 41~~ wherein said component is the calcineurin-A catalytic subunit.

WO 99/50410

PCT/AU99/00220

- 106 -

43. A method of modulating cellular functional activity in a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the expression of a nucleotide sequence encoding *Csl* or sufficient to modulate the activity of *Csl* wherein said *Csl* expression product or *Csl* modulates the activity of one or more components of a calcineurin-dependent signalling pathway.

44. A method according to claim 43 wherein said cellular functional activity is muscle cell activity.

45. A method according to claim 43 ~~or 44~~ wherein said component is the calcineurin-A catalytic subunit.

46. A method of treating a mammal said method comprising administering to said mammal an effective amount of an agent capable of modulating the expression of *Csl* for a time and under conditions sufficient to modulate muscle cell functional activity.

47. A method of treating a mammal said method comprising administering to said mammal an effective amount of an agent capable of modulating the activity of *Csl* for a time and under conditions sufficient to modulate muscle cell functional activity.

48. A method of treating a mammal said method comprising administering to said mammal an effective amount of a protein according to ~~any one of claims 15-25~~ ^{claim 15} or a derivative, homolog, analog, chemical equivalent or mimetic thereof for a time and under conditions sufficient to modulate muscle cell functional activity.

49. A method of treating a mammal said method comprising administering to said mammal an effective amount of a nucleic acid molecule according to ~~any one of claims 1-14~~ ^{claim 1} or a derivative, homolog, analog, chemical equivalent or mimetic thereof for a time and under conditions sufficient to modulate muscle cell functional activity.

00647019-042601

WO 99/50410

PCT/AU99/00220

- 107 -

claim 46

50. A method of treating a mammal according to ~~any one of claims 46 to 49~~ wherein said mammal is suffering from diabetic cardiomyopathy, cardiac hypertrophy (acquired or familial), heart failure, dilated cardiomyopathy, myocarditis, loss of myofibers and loss of regenerative capacity in ageing and skeletal myopathies such as Duchenne muscular dystrophy and Becker's myotonic dystrophy or myofiber atrophy.

claim 46

51. A method according to ~~any one of claims 46 to 50~~ wherein said mammal is administered with a combination of Csl and IGF-I or derivative, homolog, analog, chemical equivalent or mimetic of said Csl and/or IGF-I.

52. Use of an agent capable of modulating the expression of Csl or a derivative, homolog, analog, chemical equivalent or mimetic thereof in the manufacture of a medicament for the modulation of muscle cell functional activity.

53. Use of an agent capable of modulating the activity of Csl or a derivative, homolog, analog, chemical equivalent or mimetic thereof in the manufacture of a medicament for the modulation of muscle cell functional activity.

54. Use of Csl or Csl or a derivative, homolog, analog, chemical equivalent or mimetic thereof in the manufacture of a medicament for the modulation of muscle functional activity.

55. An agent for use in modulating Csl or a derivative, homolog, analog, chemical equivalent or mimetic thereof wherein modulating said Csl modulates muscle cell functional activity.

56. An agent for use in modulating Csl expression or a derivative, homolog, analog, chemical equivalent or mimetic thereof wherein modulating expression of said Csl modulates muscle cell functional activity.

05647019-012604

WO 99/50410

PCT/AU99/00220

- 109 -

65. A method of diagnosing or monitoring a mammalian disease condition, which disease condition is characterized by aberrant muscle cell functional activity, said method comprising screening for Csl or CsI in a biological sample isolated from said mammal.

09647049-043604
T093T0-6T04960